Attorney Docket # A35306-A 069906.0161 Inventors: Mitchell et al. Title: SPLICEOSOME MEDIATED RNA TRANS-SPLICING FOR CORRECTION OF SKIN DISORDERS Contact: Carmella L. Stephens (212) 408-2539 Sheet 1 of 27

FIGURE 1

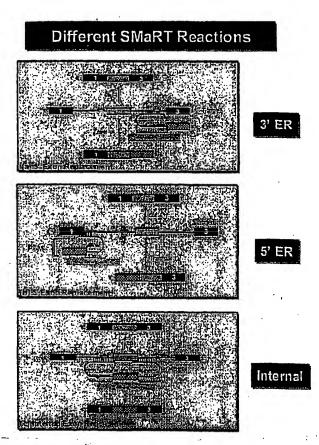


Figure 1. Schematic representation of different SMaRT reactions. (a) Trans-splicing reactions between the target 5' splice site and PIMs 3' splice site, (b) Trans-splicing reactions between the target 3' splice site and PIMs 5' splice site and (c) Replacement of internal exon by double trans-splicing reaction in which the PIM carry both 3' and 5' splice sites. BD, binding domain; BP, branchppoint sequence; PPT, polypyrimidine tract and ss, splice sites.

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2) BPAG2 Target 2 (T2)

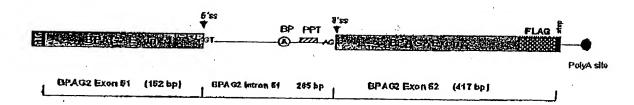


FIGURE 2B

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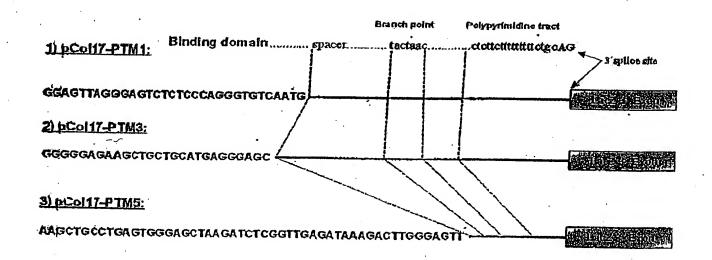


FIGURE 2C

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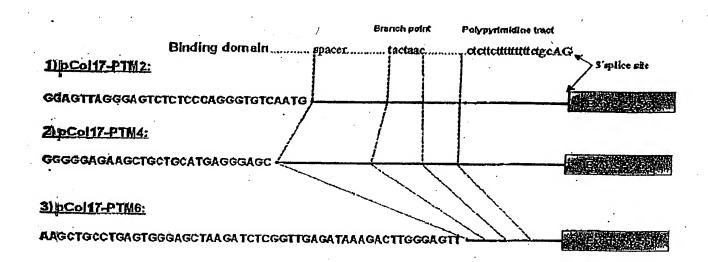
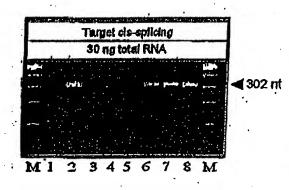


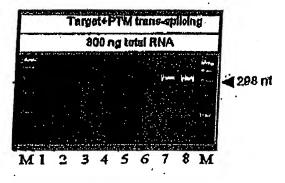
FIGURE 2D

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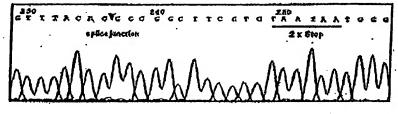
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В

sequence desplicing



sequence trans-splicing

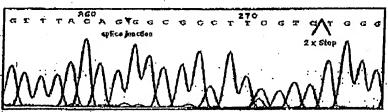


FIGURE 3A-B

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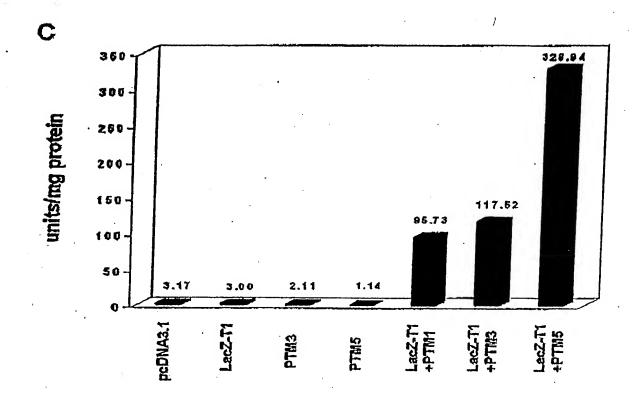


FIGURE 3C

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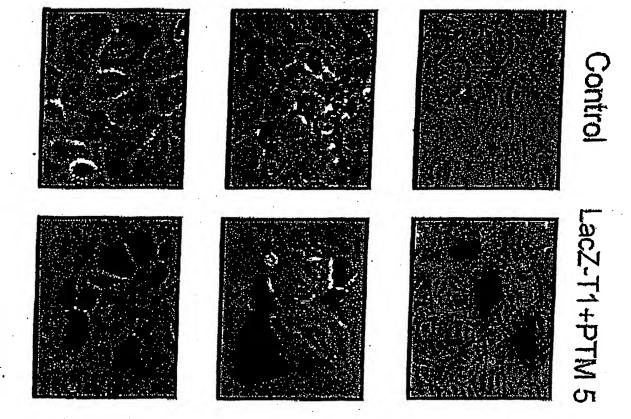


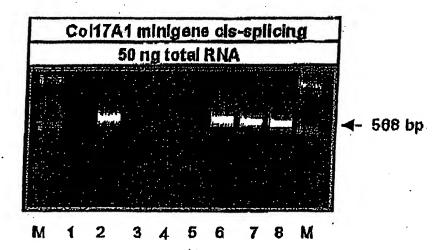
FIGURE 4

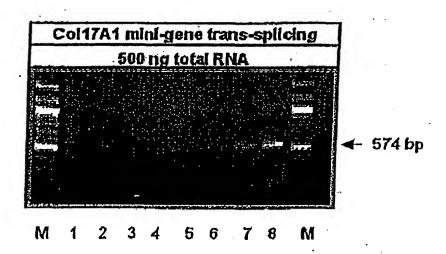
Attorney Docket # A35306-A 069906.0161 Inventors: Mitchell et al. Title: SPLICEOSOME MEDIATED RNA TRANS-SPLICING FOR CORRECTION OF SKIN DISORDERS Contact: Carmella L. Stephens (212) 408-253

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A





F

COL17A1 minigene (T2)

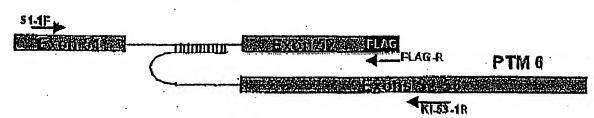


FIGURE 5A-B

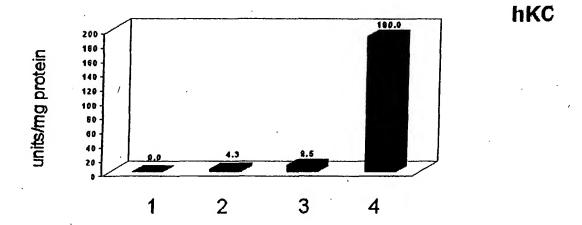
Inventors: Mitchell et al.

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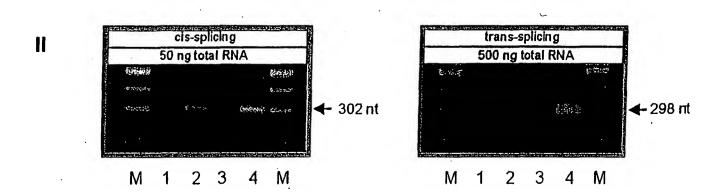
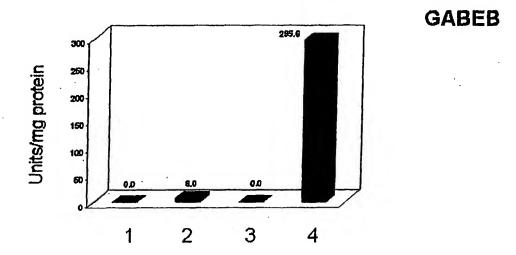
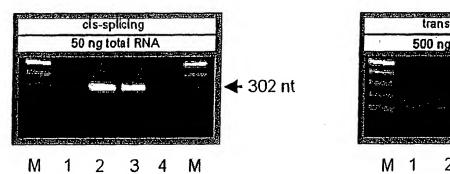


FIGURE 6A

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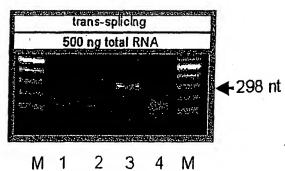
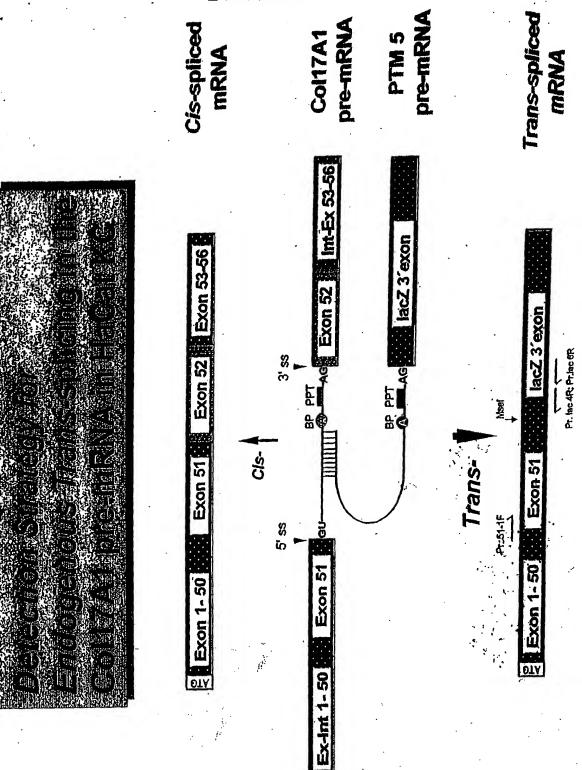


FIGURE 6B

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FIGURE 7



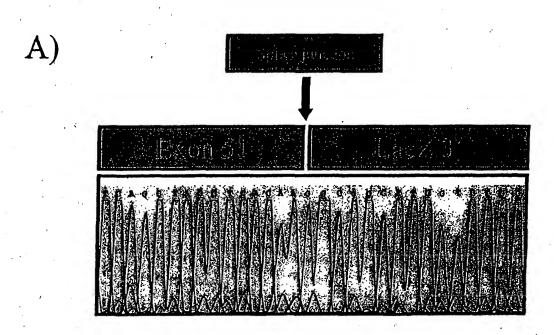
Attorney Docket # A35306-A 069906.0161 Inventors: Mitchell et al. Title: SPLICEOSOME MEDIATED RNA TRANS-SPLICING FOR CORRECTION OF SKIN DISORDERS

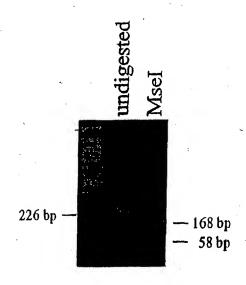
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FIGURE 8

Endogenous Transsplicing of Col17 pre-mRNA with PTM5 pre-mRNA

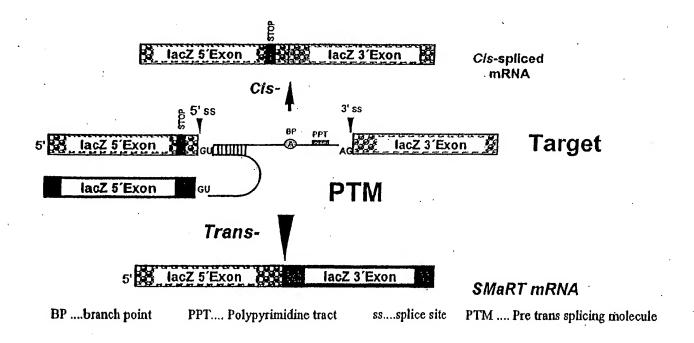




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FIGURE 9

5' trans-splicing in a LacZ-model system for hereditary diseases



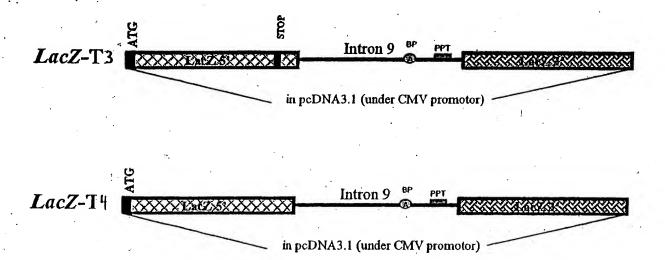
Attorney Docket # A35306-A 069906.0161 Inventors: Mitchell et al. Title: SPLICEOSOME MEDIATED RNA TRANS-SPLICING FOR CORRECTION OF SKIN DISORDERS

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FIGURE 10

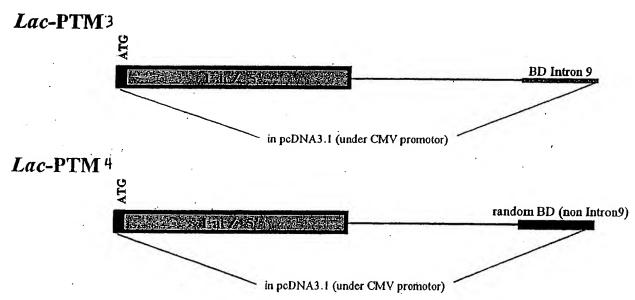
Target LacZ-T3 (containing Intron 9) and LacZ-T4 used for optimizing trans-splicing and transfection conditions



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FIGURE 11

LacZ-PTM-3 (Intron 9 specific binding domain) and LacZ-PTM-4 (non specific binding domain) for establishing optimal transsplicing conditions



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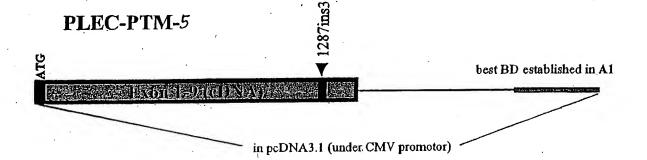
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FIGURE 12

PLEC-PTM-5 for the introduction of the 1287ins3 mutation in 293T cells

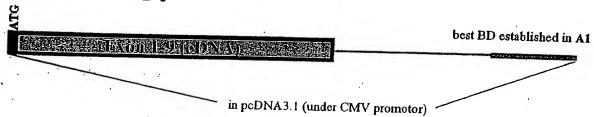


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FIGURE 13

PLEC-PTM-6 for the repair of the 1287ins3 mutation in plectin deficient patient cells

PLEC-PTM-6



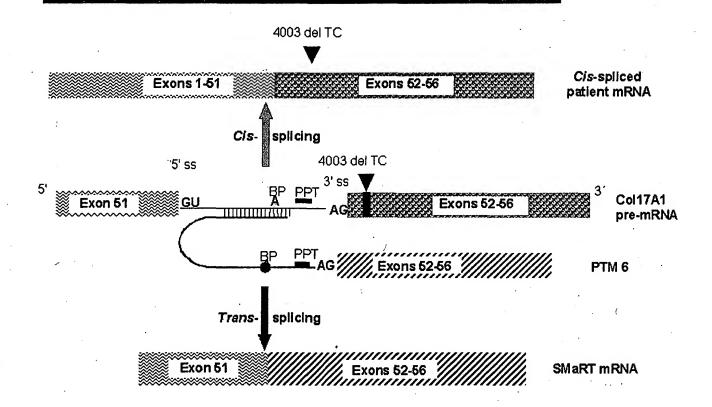
Attorney Docket # A35306-A 069906.0161
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FIGURE 14A

Trans-splicing Strategy for Col17A1 Gene (GABEB/generalized atrophic benign epidermolysis bullosa)



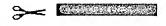
Attorney Docket # A35306-A 069906.0161 Inventors: Mitchell et al. Title: SPLICEOSOME MEDIATED RNA TRANS-SPLICING FOR CORRECTION OF SKIN DISORDERS Contact: Carmella L. Stephens (212) 408-2539 Sheet 20 of 27

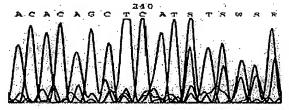
FIGURE 14B

Semi-nested RT-PCR with BPAG2-primer 51-1F, 53-1R and 52-1R

RT-PCR









Fi

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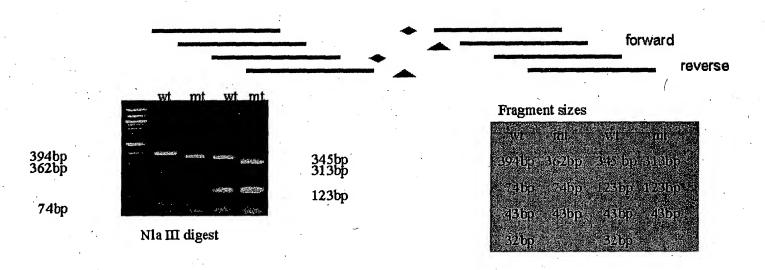
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FIGURE 14C

TOPO Cloning + Nla III digest



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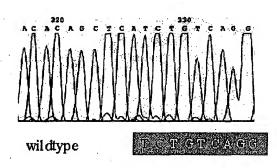
SKIN DISORDERS

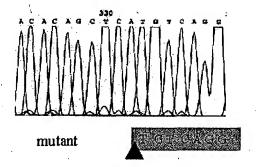
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FIGURE 14D

Sequencing of mutant and wildtype clones confirmed the correct *trans*-splicing of PTM6 into GABEB cells





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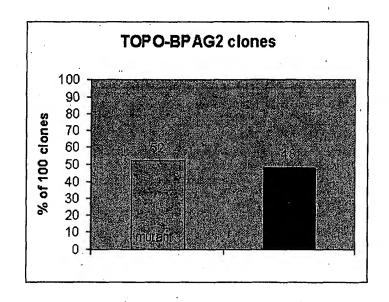
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FIGURE 14E

Analysis of 100 clones



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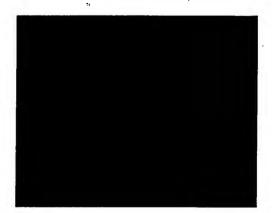
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FIGURE 15

Immunofluorescence of PTM6transfected GABEB cells

GABEB

non-transfected



GABEB

PTM6 transfected, 3 days

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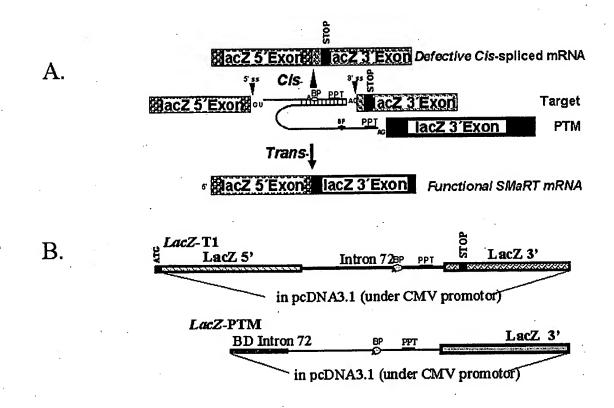
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FIGURE 16A-B

Trans-splicing strategy for COL7A1 gene (Dystrophic epidermolysis bullosa)



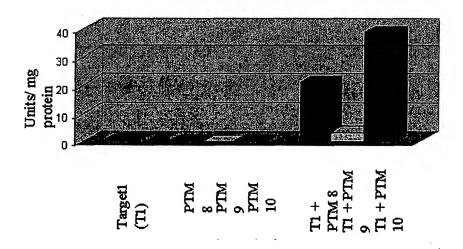
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FIGURE 17

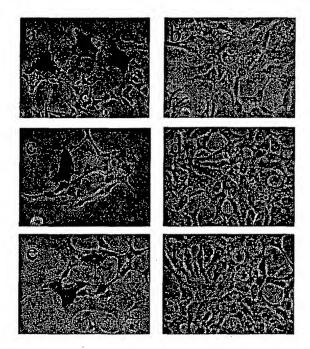
B-gal Assay of a Cotransfection of Col7 Target T1 and PTM 8, 9, 10 in 293T cells



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FIGURE 18

Beta-gal staining of Col7 PTM transfected human 293T cells



left panel: (a) Target + PTM8, (c) Target + PTM9, (e) Target + PTM10; right panel: corresponding control PTM without Target.